

AMENDMENTS TO THE CLAIMS

1. (currently amended) A method of producing packaged alphavirus particles, comprising the steps of:

(a) transfecting a first set of cells with (i) a Sindbis virus replicon comprising a sequence encoding a nonstructural protein nsp2 that comprises an amino acid selected from the group consisting of proline, leucine, glycine and valine at amino acid position 726; (ii) a first helper RNA comprising a sequence encoding an alphavirus capsid protein and cis-acting elements that allow efficient replication and packaging of said first helper RNA and (iii) a second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2 and cis-acting elements that allow efficient replication and packaging of said second helper RNA;

(b) obtaining a primary stock of viral particles comprising (i) viral particles containing said Sindbis virus alphavirus replicon[[s]], (ii) viral particles containing said first helper RNA comprising a sequence encoding an alphavirus capsid protein, and (iii) viral particles containing said second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2;

(c) infecting a second group of cells with said primary stock of viral particles at high multiplicity of infection; and

(d) obtaining a ~~high titers of~~ larger secondary stock of packaged viral particles comprising (i) viral particles containing said ~~Sindbis virus alphavirus~~ replicon[[s]], (ii) viral particles containing said first helper RNA ~~comprising a sequence encoding an alphavirus capsid protein~~, and (iii) viral particles containing said second helper RNA, wherein said secondary stock has a titer of packaged replicons at least 1×10^8 infectious units/ml ~~comprising a sequence encoding the alphavirus glycoproteins E1 and E2.~~

2. (currently amended) The method of claim 1, further comprising the steps of:

(a) infecting a ~~third~~ said second group of cells with said secondary stock of viral particles at a high multiplicity of infection; and

(b) obtaining a larger stock ~~high titers~~ of viral particles comprising (i) viral particles containing said ~~Sindbis virus alphavirus~~ replicon[[s]], (ii) viral particles containing said first helper RNA ~~comprising a sequence encoding an alphavirus capsid protein~~, and (iii) viral particles containing said second helper RNA, wherein said

~~stock has a titer of packaged replicons at least 1×10^8 infectious units/ml comprising a sequence encoding the alphavirus glycoproteins E1 and E2.~~

3. (currently amended) The method of claim 1, wherein said ~~Sindbis virus~~ ~~alphavirus~~ replicon and said first and second helper RNAs are delivered to the cells in plasmid form or in RNA form.

4. (canceled)

5. (currently amended) The method of claim 1, wherein ~~sequences encoding~~ said capsid protein and glycoproteins are ~~derived~~ from an alphavirus selected from the group consisting of Sindbis virus, Venezuelan Equine encephalitis virus, Ross River virus, and Semliki Forest virus.

6. (currently amended) The method of claim 1, wherein said cis-acting elements comprise tRNA^{Asp} and ~~the~~ replicational enhancer of Sindbis virus.

7. (canceled)

8. (currently amended) A method of generating a ~~high titer of packaged alphavirus Sindbis virus replicons-containing viral~~ particles for large-scale production of recombinant protein, comprising the steps of:

(a) transfecting a first group of cells with (i) ~~a Sindbis virus an alphavirus~~ replicon comprising a sequence encoding a heterologous protein and a sequence encoding a nonstructural protein nsp2 that comprises an amino acid selected from the group consisting of proline, leucine, glycine and valine at amino acid position 726; (ii) a first helper RNA comprising a sequence encoding an alphavirus capsid protein and cis-acting elements that allow efficient replication and packaging of said first helper RNA and (iii) a second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2 and cis-acting elements that allow efficient replication and packaging of said second helper RNA;

(b) obtaining a primary stock of viral particles comprising (i) viral particles containing said ~~Sindbis virus alphavirus~~ replicons comprising a sequence encoding said heterologous protein, (ii) viral particles containing said first helper RNA ~~comprising a sequence encoding an alphavirus capsid protein~~, and (iii) viral particles

containing said second helper RNA ~~comprising a sequence encoding the alphavirus glycoproteins E1 and E2;~~

(c) infecting a second group of cells with said primary stock of viral particles at high multiplicity of infection;

(d) obtaining ~~high titers of~~ a secondary, ~~larger~~ stock of viral particles comprising (i) viral particles containing said ~~Sindbis virus alphavirus~~ replicons comprising a sequence encoding said heterologous protein, (ii) viral particles containing said first helper RNA ~~comprising a sequence encoding an alphavirus capsid protein,~~ and (iii) viral particles containing said second helper RNA, ~~wherein said secondary stock has a titer of packaged replicons of at least 1×10^8 infectious units/ml comprising a sequence encoding the alphavirus glycoproteins E1 and E2;~~ and

(e) infecting host cells with the viral particles of (d) ~~to produce in a large scale production of~~ said heterologous protein encoded by said ~~viral particles packaged replicons.~~

9. (original) The method of claim 8, wherein said host cells are mammalian cells or insect cells.

10. (currently amended) The method of claim 8, wherein said ~~Sindbis virus~~ alphavirus replicon and said first and second helper RNAs are delivered to the cells in plasmid form or in RNA form.

11. (canceled)

12. (currently amended) The method of claim 8, wherein sequences encoding said capsid protein and glycoproteins are derived from an alphavirus selected from the group consisting of Sindbis virus, Venezuelan Equine encephalitis virus, Ross River virus, and Semliki Forest virus.

13. (currently amended) The method of claim 8, wherein said cis-acting elements comprise tRNA^{Asp} and ~~the~~ replicational enhancer of Sindbis virus.

14. (canceled)

15. (new) The method of claim 1, wherein said Sindbis virus replicon further comprises a structural RNA element that increases RNA translation efficiency.

16. (new) The method of claim 15, wherein said structural RNA element is a G-C rich sequence located 28 nucleotides downstream from an initiating AUG of Sindbis virus subgenomic RNA.

17. (new) The method of claim 8, wherein said Sindbis virus replicon further comprises a structural RNA element that increases RNA translation efficiency.

18. (new) The method of claim 17, wherein said structural RNA element is a G-C rich sequence located 28 nucleotides downstream from an initiating AUG of Sindbis virus subgenomic RNA.